The Anæsthetic and Lethal Quantity of Chloroform in the Blood of Animals.

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(From the Physiological Laboratory of the University of London.)

Observations made *in vitro* on the relations which exist when liquid chloroform and defibrinated blood are in contact have shown that with equal concentrations of chloroform the tension of this in blood, serum or solutions of hæmoglobin, is very much lower than in water or saline solutions; a definite quantity of chloroform is associated with some constituent or constituents of the blood. When known weights of chloroform and blood are mixed together at 37° C., a percentage of 1·5 or more produces a precipitate of hæmoglobin. Determinations of the quantity of chloroform held by defibrinated blood have shown that for a given weight of chloroform this amount cannot be recovered by any of the methods which have been employed. The deficit may range from 2 to 20 per cent. We are of opinion that no experiment made *in vitro* can be regarded as an indication of what obtains when chloroform vapour is inhaled. The normal physiological conditions are not reproduced even when the vapour is in contact with defibrinated blood, which is apparently not the mode in which the majority of the experiments with which we are acquainted have been conducted, and still less are physiological conditions preserved when liquid chloroform is shaken up with blood.

Our observations were commenced in October, 1905, and carried out in the Physiological Laboratory of the University of London. We have employed an entirely new method of chloroform determination applied to the blood of anæsthetised animals—large cats were used for the majority of the experiments since the phenomena of anæsthesia in these animals closely resemble those in man (MacWilliam)*—and shall only refer briefly to the methods and results of those observers who have carried out such determinations of the quantity of chloroform found in the blood, with anæsthetic and lethal doses of this drug, as can be fairly compared with the results we have obtained.

* 'Brit. Med. Journ.,' 1902.

In 1860, Lallemand Perrin and Duroy* investigated the quantity of chloroform in the blood and tissues of dogs killed with the anæsthetic. The chloroform in the blood was cleared out of this by heating in a current of air drawn through the blood, and the vapour was conducted through a red-hot glowing porcelain tube. The hydrochloric acid so formed was estimated by titration. They found 100 grammes of blood contained 1 c.c. of chloroform vapour.

In 1883, Gréhant and Quinquaud† published seven observations in which they determined the quantity of chloroform in the blood of dogs at the moment of anæsthesia, which was induced by the inhalation of 10 per cent. of chloroform vapour in air supplied by an apparatus which they had designed for administering variable amounts of chloroform. In their method the blood is removed without coming in contact with the air, and introduced into an apparatus for distilling *in vacuo*. At a temperature of 40° C., the blood gases are evolved, and at 65° C. the chloroform is distilled off. They state that almost all the chloroform in the blood is evolved as vapour with the blood gases. The receiver with the vapour and gases is washed out four to five times with water and added to the distillate. This liquid is sealed up in a tube, from which all the oxygen had been displaced by carbon dioxide, with Fehling's solution and heated. The amount of reduction was estimated by ascertaining the quantity of chloroform and water, a known weight of chloroform being used, which produced a similar amount of reduction of the same quantity of Fehling's fluid.

The quantities of blood used for these determinations were about 90 to 96 c.c., and the amount of chloroform in 1800 to 2181 grammes of blood was found to be 1 gramme. They concluded that about 50 milligrammes of chloroform per 100 grammes of blood is the anæsthetic dose, and this is only slightly less than the amount present in animals killed with chloroform.

Pohl in 1890‡ used a slight modification of the method which Schmiedeberg,§ who had adversely criticised the method employed by Lallemand Perrin and Duroy, introduced in 1867. Working with defibrinated blood and liquid chloroform, he determined the chloride produced when chloroform vapour is passed over glowing lime at a red heat. The calcium chloride is washed out of the tube with nitric acid, the liquid is neutralised with litmus and titrated with silver nitrate. The method

* 'Du Rôle de l'Alcool et des Anesthésiques dans l'Organisme,' Paris, 1860.

† 'Comptes Rendus des Séances de l'Académie des Sciences,' 9, vol. 97, p. 753, 1883.

‡ 'Arch. f. exp. Path. u. Pharmak.,' vol. 28, 1890–91.

§ 'Arch. f. Heilkunde,' p. 273, 1867.

employed by Pohl for determining the quantity of chloroform in blood during narcosis is similar, except that magnesia was substituted for lime.

The chief results of Pohl's determinations are that pure water in contact with chloroform takes up 0·794 per cent. at 15° C., which closely approximates to the figure obtained by Chancel and Parmentier,* 0·987 at 0° C., but is higher than the figures obtained by Moore and Roaf,† who find that at 17° C., with 0·04957 per cent. of chloroform vapour in a space where the vapour exerts a pressure of 74·61 mm. of mercury, the percentage by weight of chloroform taken up by distilled water is 0·586; that the amount recoverable from the blood of anæsthetised animals is much less than this, namely, 0·029, 0·018, 0·05 per cent. of chloroform, and that solutions of hæmoglobin in water do not take up more chloroform than the same volume of water, though an alcohol and ether extract of blood takes up as much as 1·105 per cent.

During narcosis in dogs Pohl obtained the following figures for arterial blood:—

	Grammes of $CHCl_3$ per 100 grammes of blood.
5 mins. after the commencement of administration ...	0·009
8 „ „ „ „ ...	0·018
60 „ „ „ „ ...	0·026
Death from chloroform	0·035
Blood from right ventricle	0·058
„ left ventricle	0·027

In another experiment in which both the cardiac and respiratory movements had ceased in a dog weighing 32 kilogrammes, in the blood of the right ventricle, 0·042 gramme per 100, and in the blood of the left ventricle, 0·058 gramme per 100 of chloroform were found.

The chief conclusions which Pohl drew from his experiments were that:—

(1) The anæsthetic dose of chloroform in the arterial blood of dogs was 0·029 or 0·018 or 0·05 gramme of chloroform per 100 grammes of blood;

(2) The average amount which was present in the blood of animals killed with chloroform was 0·035 gramme per cent.

(3) The lethal and anæsthetic dose of chloroform closely approximate one to the other.

* 'Comptes Rendus,' vol. 100, p. 773, 1885.

† 'Roy. Soc Proc.,' vol. 73, May 5, 1904.

The two experiments he records, which show the amounts of chloroform in the brain and blood of dogs killed with chloroform, are not concordant:—

	Grammes of chloroform in blood.	Grammes of chloroform in brain.
I	0·015	0·0418
II	0·043	0·036

The methods and mode of conducting the experiments detailed in Papers I and IV (appendices to the Report of the Special Chloroform Commission of the British Association)* do not come into consideration here since the work was chiefly undertaken with the view of ascertaining how far weighed amounts of liquid chloroform could be recovered from the tissues of small animals (Dodgson) or from the blood of animals anæsthetised or killed by chloroform, the blood being examined quantitatively for chloroform at periods varying from 1 to 10 days after the blood had been removed from the body (J. H. Wells).

Since the recent work of Tissot† and Nicloux‡ is directly comparable with that which we have undertaken, their method and results will be given in somewhat greater detail. The method of the French observers J. Mansion and Tissot,§ and also that employed by Nicloux, depends on Dumas' reaction, the production of potassium chloride when chloroform is treated with alcoholic potash. As applied to the estimation of chloroform in urine or blood outside the body, or blood withdrawn from different parts of the circulation, the method consists in distilling off the chloroform from defibrinated blood or other liquid which has been rendered acid with alcoholic solution of tartaric acid. The distillate is boiled with alcoholic potash, cooled, neutralised with sulphuric acid, using phenolphthalein as an indicator, and titrated with silver nitrate, using potassium chromate as an indicator (Nicloux). The error of the method averages 2 per cent. (Nicloux), while Tissot and Mansion show that 96·1 per cent. of the total chloroform added can be obtained from blood treated as above. The method adopted of distilling off chloroform from tissues after acidification is the usual toxicological method. One of us (J. A. G.) has been in the habit of frequently using this method of investigation in toxicological practice, and in his experience every trace of chloroform cannot be readily extracted and accurately estimated when only a few milligrammes are present. Without wishing to impugn in the slightest degree the accuracy of the control experiments of Nicloux, we are of opinion that the exact

* 'Brit. Med. Journ.,' July 11, 1902.

† 'Comptes Rendus,' No. 5, 1906.

‡ Nicloux, 'Comptes Rendus,' No. 2, 1906; No. 3, 1906; No. 7, 1906.

§ J. Tissot, 'Comptes Rendus,' No. 4, 1906.

calculated and theoretical values found when working with 4 and 5 milligrammes of chloroform in 100 grammes of blood are due to accidental coincidence, more especially when we remember that the analytical process used involves distillation and subsequent saponification with alcoholic potash. We would also urge that even if it be granted as possible to estimate exactly by this method amounts of chloroform mixed with blood *in vitro*, the same accuracy cannot be expected with chloroform which has been introduced into the blood by the physiological process of inhalation; at the same time we must admit that his figures do not greatly differ from those in our experiments.

The results obtained by Nicloux show for dogs:—

(1) That the anæsthetic dose varies for different animals.

(2) The amount in the arterial blood when anæsthesia is induced is about 50 milligrammes per 100 grammes of blood (confirmatory of Gréhant's results). In a dog weighing 18 kilogrammes, 58·5 milligrammes were found 60 minutes after commencement of administration, in another weighing 9·3 kilogrammes it was 47 milligrammes 33 minutes after the commencement of administration.

(3) The lethal dose in venous blood may be 70, 69, or 73 milligrammes per 100 grammes of blood.

(4) During the pre-anæsthetic period the intake of chloroform is rapid even with a low percentage of chloroform in the inspired air. Thus, within three minutes 56 milligrammes were found with a high percentage of chloroform in the air, and 55·5 milligrammes at the end of 32 minutes with a small percentage of chloroform.

(5) The differences between amounts of chloroform in arterial blood with an anæsthetic and lethal dose are small.

Anæsthetic dose. Milligrammes per 100 grammes.	Lethal dose. Milligrammes per 100 grammes.
54	70
57	64
57	69

An examination of Nicloux's figures, however, shows that in some cases the lethal dose was less than the anæsthetic one for the same dogs, thus—

Anæsthetic dose. Milligrammes per 100 grammes.	Lethal dose. Milligrammes per 100 grammes.
47	42
48	41

This is possibly due to alterations in the rate and depth of respiration. The weights of the dogs show that there is no relation between weight and an anæsthetic dose of chloroform—

	Anæsthesia.
Weight in kilogrammes.	Milligrammes per 100 grammes.
23	54
8	50
18	58
9	57

After anæsthesia has been induced and the supply of chloroform is stopped, this drug rapidly leaves the blood. Five minutes after anæsthesia half the amount disappeared; three hours later 7 milligrammes were found in the blood, which became free from chloroform seven hours after narcosis.

Using centrifugalised oxalated blood removed from the inferior vena cava, Nicloux recovered 64·4 per cent. of the chloroform from the red corpuscles and 13·3 per cent. from the plasma. However, the volume of the corpuscles was high in both the experiments he has described, being in one case 14 c.c. of plasma to 26 c.c. of deposit, and in the other 15 c.c. of plasma to 25 c.c. of corpuscles. His figures of the distribution of chloroform in blood are much below those of Pohl, who has stated that 87 to 90 per cent. of the total chloroform is held by the red corpuscles, that is the chloroform-holding power of the corpuscles is 7 to 8 times that of the plasma. The latter figures, as will be shown subsequently, are more in accord with our own observations.

The results obtained by J. Tissot show that:—

(1) 34 to 40 milligrammes of chloroform in 100 grammes of blood is sufficient to produce anæsthesia (Tissot), or 32 to 43 milligrammes (Mansion and Tissot).

(2) When mortal syncope is produced in dogs with very slow inhalation of small percentages of $CHCl_3$, the following amounts of chloroform are found in arterial blood:—

Milligrammes per 100 grammes.	Milligrammes per 100 grammes.
96	60
88·1	67·2
84·2	105·2
59·8	77·1

Just prior to mortal syncope the amount of chloroform in blood falls thus:—

Milligrammes per 100 grammes.	Milligrammes per 100 grammes.
65	62·2
62·5	56·05
54·5	

Tissot's deduction from this diminution in the blood which is constant just before death is that the choroform of the blood passes into and accumulates in the tissues, especially in the brain.

(3) The amount of chloroform in blood which produces anæsthesia varies with the rate of the induction of anæsthesia.

Mins.	Milligrammes of $CHCl_3$ per 100 grammes of blood.
2—3	60—70
5—8	44
Much slower	34, 35

(4) The determinations of the amount of chloroform found in venous blood after death by Nicloux is, according to Tissot, useless for a determination of the lethal dose, for this is always less than what is found in arterial blood, and when dogs are killed by the slow inhalation of 5, 6, 7, or 8 per cent. of chloroform vapour the lethal figure for arterial blood varies between 48 and 67 milligrammes. A quantity as large as 160 milligrammes may be found in blood before respiration stops. Such a large accumulation is impossible with inhalation of 4 to 5 per cent. chloroform vapour, for with this amount of chloroform it is impossible to raise the amount in arterial blood above 40 to 45 milligrammes per 100 grammes.

(5) Anæsthesia can exist with a very low content in the blood, for instance, 29 milligrammes per 100.

(6) There is no relation whatever between the proportion of chloroform in the blood and the effects of the drug, for these are determined, not by the absolute amount of chloroform present in blood, but by the quantity which enters the central nervous system. The amount in this may equal, never exceeds, and generally is much below that in the blood.

(7) During recovery from anæsthesia it has been constantly proved that the amount in venous blood exceeds that in the arterial. A study of the amount of chloroform in arterial blood should be made during the induction of anæsthesia and of the amount in venous blood during the disappearance of this condition.

	Cessation of chloroform administration immediately after anæsthesia.	45 minutes later.	2 hours later.
Arterial blood ...	53·2	5·8	0
Venous blood ...	48·1	7·7	4·9

The effect of chloroform upon the blood, which precipitates hæmoglobin and

certain proteids of the serum (Formánek),* or the hæmolytic effect described by Harley† in 1865 with 5 per cent. of chloroform in blood, we have never observed in any of our experiments, since both the anæsthetic and lethal doses lie far below the quantity required to produce those effects.

Gréhant originally recognised that a combustible gas, which he believed was carbon monoxide, could be extracted from blood, and, in 1898, Desgrez and Nicloux‡ stated that normal blood contains 1 c.c. per cent. of total gases other than the normal, which they considered was carbon monoxide. During anæsthesia extending over five hours, the amount of this gas was found to be 2·5 c.c. per 1000 grammes of blood. Their recognition of this gas depends on the fact that carbon monoxide oxidised by anhydrous iodic acid at 150° C. yields carbon dioxide and free iodine. The latter is recovered, dissolved in chloroform and the tint of the solution estimated against a standard scale. Three experiments are described and from these it is concluded that chloroform is actually decomposed within the organism. We have obtained no evidence that any gas of this nature appears during chloroform narcosis, and since at least 80 per cent. of unchanged chloroform can be extracted from shed blood, it appears improbable that the remaining amount of chloroform in 1000 grammes of blood could yield anything like the quantity of 2·5 c.c. of carbonic oxide gas. They further state that in intense chloroform narcosis the proportion of carbonic oxide may even reach 6·9 c.c. per litre of blood.

If the contention of Desgrez and Nicloux is correct that as much as 6·9 c.c. of carbon monoxide may appear in 1000 grammes of blood during intense chloroform narcosis, about 1/25 of the total hæmoglobin would be saturated with this gas. The following experiment was made to determine whether any of the hæmoglobin is in the state of CO-hæmoglobin :—

Cat. Weight 3 kilogrammes.

2.30 P.M. Ether anæsthesia commenced.

3 „ 4 c.c. of blood withdrawn from carotid artery and defibrinated.

3.6 „ Chloroform inhalation commenced, the vapour being given by air drawn over liquid chloroform in a Woulff's bottle.

4.20 „ Anæsthesia with chloroform finished, inhalation pushed until respiration ceased. Blood to the amount of 75 c.c. was withdrawn and defibrinated. This was intensely dark.

* Formánek, 'Zeitschr. f. physiol. Chemie,' vol. 29, p. 416, 1900.

† 'Phys. Soc. London Proc.,' 1865.

‡ 'Comptes Rendus,' p. 274, 1898.

No difference whatever could be detected, either spectroscopically or by using Haldane's method of examining very dilute solutions of blood in long glass tubes, between the condition of the hæmoglobin before and after $1\frac{1}{4}$ hours' continuous chloroform narcosis.

However, positive information that CO-hæmoglobin is present can be at once obtained by the above methods when 0·5 c.c. pure CO was added and shaken up with 73 c.c. of the same blood which had failed to give any indication of CO-hæmoglobin. We are therefore unable to accept the view that the combustible gas stated to appear during chloroform narcosis is carbon monoxide, a product of the decomposition of chloroform within the organism. The recognition of CO-hæmoglobin is quite easy when 1/10 to 1/20 of the total colouring matter of the blood has taken up carbon monoxide.

I.—*Determination of the Amount of Chloroform in Blood.*

In the experiments described in this paper the amount of chloroform in the blood was calculated from the difference in the chlorine-content of the blood before and after administration of the anæsthetic. The validity of this mode of estimation depends, of course, on the constancy of the natural chlorine-content of the blood during the whole course of an experiment. We fully satisfied ourselves, however, by a series of preliminary observations, some of the results of which are given later, that under the conditions of all our experiments the percentage of natural chlorine did remain sufficiently constant.

Method.—The animal experimented on was anæsthetised by ether and the necessary operations for introducing cannulæ into the carotid artery and the trachea were performed. The tracheal cannula was fitted to a Chauveau's valve and a side-tube in connection with apparatus for recording the rate and character of the respirations. Chloroform was administered sometimes by means of a Woulff's bottle, sometimes from bags filled with mixtures of air and chloroform vapour of known composition by means of the Dubois apparatus. The samples of blood were taken from the carotid artery and collected in stoppered weighing tubes. As a rule the ether was cut off after the operation had been performed, and the animal allowed so far to recover that the conjunctival reflexes reappeared and the anæsthesia was light. A control sample was then taken in order to determine the percentage of natural chlorine. After this, chloroform was administered and samples of blood were taken during various stages of anæsthesia. No blood-pressure observations were taken, as we thought it desirable to interfere as little as possible with normal physiological conditions. During the experiments

on any particular animal, which sometimes lasted two hours or longer, every effort was made, by keeping a close watch on the animal, to prevent any sudden fluctuations in the rate of administration—for instance, if the animal held its breath the chloroform was not pushed. In no experiment did the animal lose any blood beyond what was taken for the samples, except in certain experiments deliberately undertaken to test the effect of loss of blood.

Mode of Estimating the Chlorine.—After careful consideration of the various available methods for estimating chlorine, we selected, owing to the volatility of chloroform, the well-known method of Carius, commonly used for the determination of the amount of halogen in organic compounds. The stoppered weighing tube, containing, as a rule, from 5 to 6 grammes of blood, was placed along with 6 to 8 c.c. of fuming nitric acid (1·5), and an excess of solid silver nitrate in a bomb tube, which was then sealed. The bomb was then heated in the furnace to 150° C. for six hours. With the quantities of material used, this time and this temperature proved quite sufficient to completely oxidise the organic matter. The bomb was then opened and the silver chloride formed was weighed. It is generally considered that chlorine can be estimated with great exactness by the gravimetric method, using paper filters and, according to Fresenius, we can, with care, always obtain 99·9 to 100·1 for 100 parts of chlorine taken. By adopting Gooch's method of filtration and J. P. Cooke's suggestion of washing with water containing a little silver nitrate, by which the small minus error caused by the slight solubility of silver chloride in water is obviated, and a few other simple precautions, a much higher degree of accuracy can be attained.

As the quantities of silver chloride weighed in our experiments were very small—varying from 0·03 to 0·09 gramme—we thought it necessary to adopt all possible precautions to obviate the slight errors, which, when large quantities are weighed, are for ordinary practical purposes negligible.

The contents of the bomb tube were transferred to a beaker, diluted with water and heated to the boiling temperature for a few minutes. After cooling, the liquid was filtered through the Gooch, and the silver chloride washed into the crucible by means of hot water containing a little nitric acid and silver nitrate. The washing was completed with cold water. The crucible and its contents were then dried at 140° C. and weighed. The silver chloride was then dissolved on the filter by means of strong ammonia, and the asbestos washed with ammonia until free from silver. The crucible was then finally washed with hot water, dried as before, and weighed. The difference between the two weighings, (1) crucible + silver chloride + possible glass fragments and (2) the crucible + fragments, gave the absolute amount of chloride present.

It was found more convenient to obtain the weight of the silver chloride in this way, rather than directly by filtering through an already weighed crucible, as it was almost impossible to prevent traces of glass dust getting into the silver chloride during the heating and during the opening of the bomb. This plan was, therefore, adopted in many of the experiments, but in any case the result was always checked by dissolving out the silver chloride in the manner described. We believe this plan far preferable in our case to that usually followed of dissolving the silver chloride away from the glass and then reprecipitating with nitric acid.

Control Experiments.—In order to ascertain whether the percentage of chlorine in the blood of an animal remains sufficiently constant during prolonged anæsthesia accompanied by periodical bleeding, a number of experiments were made with cats, which conclusively showed that under the conditions of our experiments the percentage does remain constant. In illustration we quote the following experiments:—

Experiment 1.—A cat, weighing 3·2 kilogrammes, was anæsthetised by ether and samples of the blood taken at intervals of ten minutes, the character of the respirations being recorded on a drum. Every effort was made to keep the anæsthetic condition of the animal as constant as possible during the experiment. It was found that the colour of the blood afforded a good index of the degree of the anæsthesia. Six samples of blood were taken, after which chloroform was administered until the animal was asphyxiated, when a seventh sample was taken. The results of the analysis are given in the following table—Table I. The colour of the blood after respiration ceased was a deep brownish-red, such as we have always found to be the case in chloroform narcosis at this stage, though the oxygen-carrying property of hæmoglobin is known not to be impaired by chloroform.

Experiment 2*a*.—A pregnant cat, weighing 3·3 kilogrammes, was anæsthetised as before, and samples of blood taken. The experiment was somewhat similar to No. 1, except that the intervals between the abstraction of the various samples were longer. Five samples were taken, after which chloroform was administered until the animal was very deeply under the influence of the drug, but was still breathing satisfactorily.

The results are given in Table IIA.

Experiment 2*b*.—A cat, weighing 2·5 kilogrammes, was etherised. Four samples of blood were collected for analysis, but between the first and second samples 10 c.c. of blood were withdrawn, and between the third and fourth 5 c.c. After the fourth sample was taken the animal was chloroformed, using a Woulff's bottle, until respiration ceased.

The results are given in Table IIB.

Table I.—April 26, 1906. Cat under Ether. Weight, 3·2 kilogrammes.

Time.	Anæsthetic.	Weight of blood in grammes.	Weight of AgCl.	Percentage of chlorine.	Mean value of chlorine, per cent.	Deviation from mean.	Average deviation from mean.	Respirations per minute.	Remarks.
P.M.									
2.15	Ether began								
2.55	Ether	5·1568	0·0692	0·33079		−0·00061		23	Reflexes just visible. Blood medium colour
3.5	„	5·2274	0·0698	0·32916		−0·00224		23	Ditto
3.15	„	4·3200	0·0583	0·33267	0·331396	+0·00127	0·001123	23	Colour slightly brighter
3.25	„	4·6081	0·0619	0·33114		−0·00026		—	Colour slightly brighter. Cheyne Stokes' respirations
3.35	„	5·2557	0·0706	0·33113		−0·00027		24	Much same
3.45	„	4·4572	0·0603	0·33349		+0·00209		24	About same
3.46	Chloroform put on, using Woulff's bottle, with entrance tube under $CHCl_3$, and continued until the animal was asphyxiated. A sample of blood was now taken, which was deep red in colour. It did not smell noticeably of chloroform							17	Reflexes gone Cessation of respiration
		6·9746	0·1035	0·36581		0·0344 as chlorine 0·0386 as chloroform			
		= 7·7 c.c. as vapour of chloroform at 0° C. and 760 mm.							

Table IIA.—Pregnant Cat. Weight, 3·3 kilogrammes. Ether.

Time.	Anæsthetic.	Weight of blood in gr.	Weight of AgCl.	Percentage of chlorine.	Mean value of chlorine, per cent.	Deviation from mean.	Average deviation from mean.	Remarks.
A.M.								
11.0	Ether started							
11.35	Ether	3·6765	0·0518	0·34732		−0·00048		1st sample taken
11.50	,,	4·5590	0·0641	0·34659		−0·00021		2nd sample, colour same
P.M.								
12.13	,,	5·2646	0·0740	0·3465	0·3478	−0·0013	0·00165	3rd sample, colour similar
12.28	,,	4·6735	0·0668	0·35244		+0·0046		4th sample, colour same
12.43	,,	6·0244	0·0846	0·34617		−0·0016		5th sample, colour same
12.44	Chloroform was now administered by means of a Woulff's bottle, and at 12.56, when the animal was fairly deeply under, a sample of blood was drawn. It was rather dark in colour.							
		5·5070	0·0821	0·3675		0·0197 as chlorine 0·0222 as chloroform		
	= 4·15 c.c. as vapour of $CHCl_3$ at 0° C. and 760 mm.							

Table IIB.—Cat. Weight, 2·5 kilogrammes.

Time.	Anæsthetic.	Weight of blood in gr.	Weight of AgCl.	Percentage of chlorine.	Mean value of chlorine, per cent.	Deviation from mean.	Average deviation from mean.	Remarks.
A.M.								
11.30	Ether started							
P.M.								
12.7	Ether	5·2180	0·0729	0·34439		+0·00304		1st sample, 10 c.c. extra drawn
12.8	,,							
12.35	,,	4·3222	0·0597	0·34049		−0·00086		2nd sample
12.50	,,	4·2139	0·0584	0·34163	0·34135	+0·00028	0·00166	3rd sample, 5 c.c. drawn for another purpose
12.57	,,							
1.14	,,	4·9247	0·0677	0·33888		−0·00247		4th sample
1.19	Chloroform was now slowly admininistered, more rapidly towards the end, by means of a Woulff's bottle, until respiration ceased. Sample was very dark in colour.							
		6·2537	0·0947	0·37329		0·03194 as chlorine 0·03586 as $CHCl_3$		
	= 6·73 c.c. as vapour of $CHCl_3$ at 0° C. and 760 mm.							

Experiment 3.—We quote this experiment as an illustration of the greatest difference in the chlorine content of the blood ever noticed. In this particular experiment a large quantity of blood, about 23 c.c., was withdrawn during the interval between the times of taking the samples analysed. The cat weighed 3·8 kilogrammes. The results are given in Table III.

Table III.—April 13, 1906. She Cat. Weight, 3·8 kilogrammes.

Time.	Anæsthetic.	Weight of blood in gr.	Weight of AgCl.	$CHCl_3$ per cent.	Mean value of chlorine per cent.	Deviation from mean.	Average deviation from mean.	Remarks.
P.M.								
12.15	Ether on							
12.48	„	4·2378	0·0572	0·33273		−0·00525		
12.50	„	—	—	—	0·33798	—	0·00525	18 c.c. blood taken from animal, making altogether about 23 c.c.
1.4	„	3·9088	0·0543	0·34323		+0·00525		Animal nearly asphyxiated. It could not be brought round

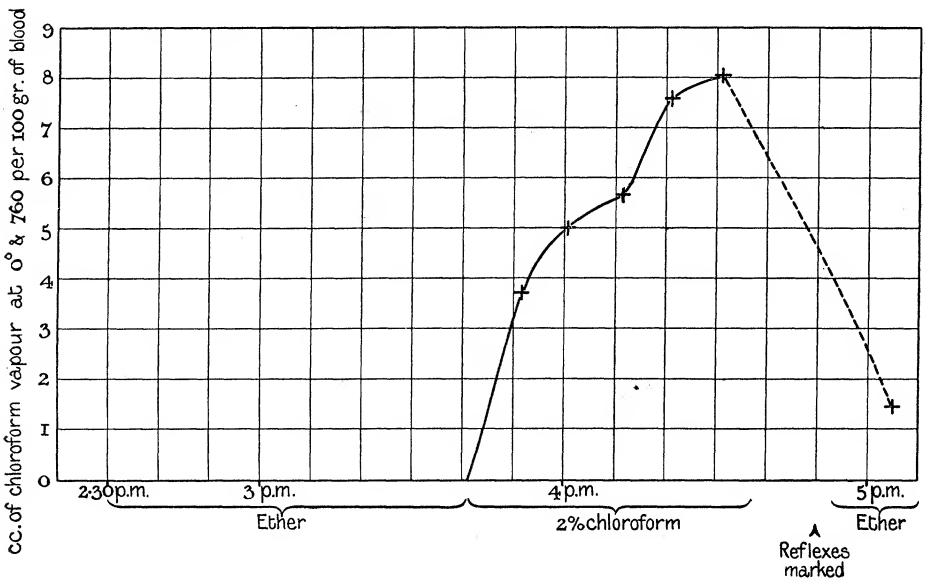
Experiments to Determine the Amount of Chloroform in the Arterial Blood of an Animal at Different Stages of Chloroform Narcosis.

Experiment 4.—This was one of the earliest experiments performed. The blood was taken from the left carotid. The chloroform was administered by means of a Woulff's bottle, and after the animal was fairly deeply anæsthetised it was kept as far as possible under constant conditions. The results are given in Table IV.

Table IV.—Cat. Left carotid tapped.

Time.	Anæsthetic.	Weight of blood in gr.	Weight of AgCl.	Percentage of chlorine.	Difference from control—			Remarks.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.	
P.M.								
12.30	Ether started							
1.0	Ether	5·0843	0·0677	0·3282	—	—	—	Sample taken when reflexes just gone
1.0½	Chloroform on							
1.9	„	3·8387	0·0534	0·3429	0·0147	0·0165	2·56	Reflexes going
1.14	„	4·5512	0·0645	0·3493	0·0213	0·024	4·5	Animal maintained in the same state of anæsthesia throughout
1.19	„	5·6276	0·0796	0·3487	0·0206	0·023	4·3	

Experiment 5.—In this experiment a male cat, weighing 4 kilogrammes, was used, and the blood was taken from the right carotid. The chloroform was administered from bags filled by means of the Dubois apparatus, with approximately a 2-per-cent. mixture of chloroform vapour and air. The animal was gradually anæsthetised until respiration ceased, when the chloroform was cut off and the cat allowed partially to recover. It was then kept under light ether anæsthesia until 5.5 P.M., 29 minutes after the chloroform was stopped, when a final sample of blood was drawn. The samples of blood while the animal was under chloroform were taken every 10 minutes, and the respirations were recorded. The darkness of the colour of the blood



A.

Constructed from Experiment 5. Samples of arterial blood (+).

increased with the percentage of anæsthetic in the blood, and appeared to afford a good criterion of the progress of the anæsthesia. The results of this experiment are given in Table V and Curve A. After the chloroform was cut off the anæsthetic was rapidly eliminated from the blood, and 14 minutes later the reflexes were well marked. At the time of taking the final sample the greater portion of the chloroform had disappeared.

Experiment 6.—For this experiment a female cat, weighing 4·3 kilogrammes, was taken. The right carotid was tapped, and the chloroform administered by means of a Woulff's bottle. The control sample was taken when the reflexes were just visible. The chloroform was then administered, and samples

Table V.—Cat. Weight, 4 kilogrammes. Chloroform administered by Dubois Apparatus. Right carotid tapped.

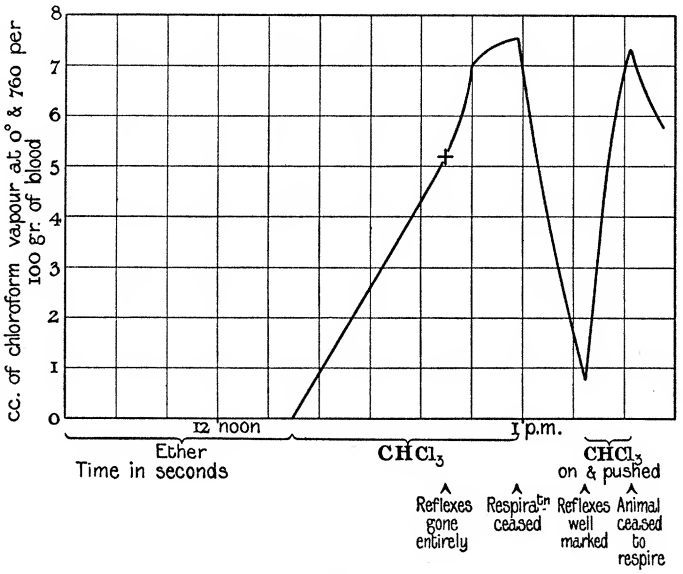
Time.	Anæsthetic.	Weight of blood in gr.	Weight of AgCl.	Per-centage of chlorine.	Differences from control—			Colour.	Respirations per minute.	Remarks.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.			
P.M.										
2. 30	Ether put on.									
3. 41	Ether off	4 ·9281	0 ·0667	0 ·3336	—	—	—	Fairly bright ...	30	Convulsive movements
3. 41	Chloroform on, 2 per cent.									
3. 51	„ „	4 ·4422	0 ·0633	0 ·3513	0 ·0176	0 ·0198	3 ·71	Slightly darker...	19	
4. 1	„ „	4 ·1523	0 ·0602	0 ·3574	0 ·0237	0 ·0266	5 ·0	„ ...	18	
4. 11	„ „	4 ·4326	0 ·0648	0 ·3604	0 ·0267	0 ·03	5 ·63	Slightly deeper...		
4 ·21	„ „	4 ·3817	0 ·0657	0 ·3696	0 ·036	0 ·0404	7 ·58	Much same	36	Deep respirations
4. 31	„ „	5 ·8127	0 ·0877	0 ·3719	0 ·0384	0 ·0431	8 ·09	Rather lighter ...	30	Sample difficult to get, squeezing resorted to
4. 36	Chloroform off.									
4. 50	.....	—	—	—	—	—	—	—	—	Reflexes marked
4. 53	Ether on.									
5. 5	Ether on	4 ·6707	0 ·0645	0 ·3404	0 ·0068	0 ·0076	1 ·4	Very bright	20	

Table VI.—Cat. Weight, 4·3 kilogrammes. Right carotid tapped. Chloroform administered from Woulff's bottle.

Time.	Anæsthetic.	Weight of blood in grammes.	Weight of AgCl.	Percentage of chlorine.	Difference from control—			Colour.	Remarks.
					As chlorine.	As chloroform.	As vapour of $CHCl_3$ at 0° C. and 760 mm.		
A.M. 11.30	Ether started								
P.M. 12.14	Under ether	5·6099	0·0754	0·3313	—	—	—	Medium.....	Reflexes just reappearing
12.14	Chloroform on instead of ether, light anæsthesia								
12.45	,, ,,	3·8512	0·0556	0·3559	0·0246	0·0276	5·17	Medium.....	Eye and tail reflexes just disappeared entirely
12.50	,, ,,	3·5211	0·0521	0·3647	0·0334	0·0375	7·04	Rather dark*	Breathing satisfactory
12.59	,, ,,	4·0755	0·0607	0·3671	0·0358	0·0402	7·55	Very dark ...	Respiration just ceased
12.59	Chloroform stopped.....	—	—	—	—	—	—	—	Artificial respiration resorted to
1.2	.....	—	—	—	—	—	—	—	Breathing began naturally
1.12	.....	4·2136	0·0573	0·3352	0·0039	0·0044	0·82	Very bright	Reflexes just reappeared
1.13	Chloroform on and pushed								
1.21	,, ,,	4·8972	0·0727	0·3659	0·0346	0·0389	7·30	Very dark ...	Animal on point of asphyxiation

The animal was brought round again by use of the bellows, and a sample of blood, taken when reflexes just reappeared, was bright.

taken when the conjunctival and tail reflexes had entirely disappeared, five minutes later, and when respiration just ceased. The chloroform was stopped at this point, and the animal allowed to recover. In this case artificial



B.

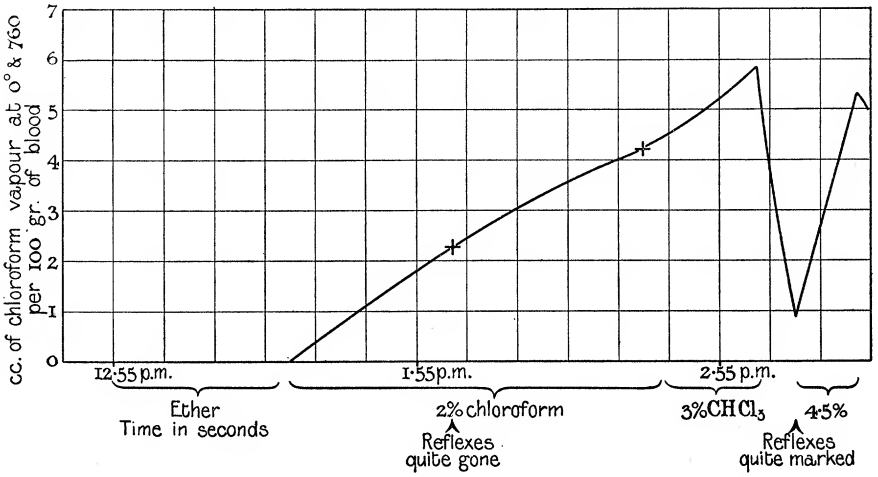
Constructed from Experiment 6.

respiration had to be resorted to before the cat began to breathe naturally. A sample of blood was collected when the reflexes had just entirely reappeared. After this stage chloroform was again administered until the animal was on the point of asphyxiation, when the final sample of blood was taken. It will be noticed that in this experiment also the colour of the blood kept pace closely with the amount of anæsthetic in the blood. After the final sample had been taken the animal was revived again by the use of the bellows, and the blood appeared bright in colour. The amounts of chloroform per cent. of blood were practically identical at the two points where respiration ceased. The results of the experiment are recorded in Table VI and Curve B.

Experiment 7.—In this case a male cat, weighing 3·5 kilogrammes, was taken and the right carotid tapped. The chloroform was administered from bags filled with mixtures of chloroform vapour and air by means of the Dubois apparatus. At first 2-per-cent. chloroform mixture was used, and samples of blood were drawn after the reflexes had entirely vanished and when the animal was very deeply under. Three-per-cent. chloroform mixture was then substituted for 2-per-cent., and a sample taken when the animal

Table VII.—Cat (male). Weight, 3·5 kilogrammes. Blood taken from right carotid. Chloroform administered by Dubois apparatus.

Time.	Anæsthetic.	Weight of blood in grammes.	Weight of AgCl.	Percentage of chlorine.	Differences from control—			Colour.	Remarks.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.		
P.M.									
12.55	Ether started								
1.27	Under slight ether	4·0709	0·0547	0·3313	—	—	—	Fairly bright...	Reflexes just reappeared
1.28	Chloroform on, 2 per cent. approximately								
1.33	Chloroform off	—	—	—	—	—	—	—	Breathing unsatisfactory
1.55	2 per cent. chloroform on								
2.2	Continued	3·9485	0·0548	0·3421	0·0108	0·0121	2·27	Slightly darker	Respiration, 20 per min.
2.21	2 per cent. continued	—	—	—	—	—	—	—	„ 18 „
2.30	„ „	—	—	—	—	—	—	—	„ 23 „
2.39	„ „	—	—	—	—	—	—	—	„ 21–22 per min.
2.40	„ „	3·8731	0·0551	0·3509	0·0196	0·0223	4·18	Deeper	
2.44	3 per cent. chloroform on								
2.48	Continued	—	—	—	—	—	—	—	Respiration shallow and very irregular, about 40 per min., evidently the Cheyne-Stokes type of breathing. This continued, and was very clearly marked
2.59	„	4·2182	0·0614	0·3588	0·0275	0·0309	5·79	Very deep	
3.2	Chloroform off	—	—	—	—	—	—	—	Animal began to die; after discontinuance of $CHCl_3$ it recovered naturally
3.10	.....	4·0592	0·0553	0·3358	0·0045	0·005	0·94	Fairly bright...	Reflexes well marked
3.11	4·5 per cent. $CHCl_3$ on								
3.15	„ „	—	—	—	—	—	—	—	Respirations, 20 per min., animal difficult to get under
3.19	„ „	—	—	—	—	—	—	—	Reflexes not quite gone, and clonic twitches of hind limbs
3.22	„ „	4·4367	0·0641	0·3561	0·0248	0·0279	5·23	Very dark indeed	Movements vanished and respiration almost ceased



C.

Constructed from Experiment 7.

showed signs of asphyxiation. During the administration of the 3-per-cent. mixture the respiration was shallow and irregular, and of the Cheyne-Stokes type. The animal was then allowed partially to revive, and a sample of blood was collected when the reflexes were well marked. Chloroform was now administered again, the mixture of chloroform vapour and air used being of approximately 4·5 per cent. strength. The reflexes took rather a long time to entirely disappear, and the time of disappearance was rendered more difficult to ascertain by the complication of clonic twitching of the hind limbs. These movements vanished before respiration ceased, when a sample of blood was collected.

The results of the experiment are given in Table VII and Curve C. It will be noticed that the amounts of chloroform in the blood at the two stages of the experiment at which respiration ceased are almost identical.

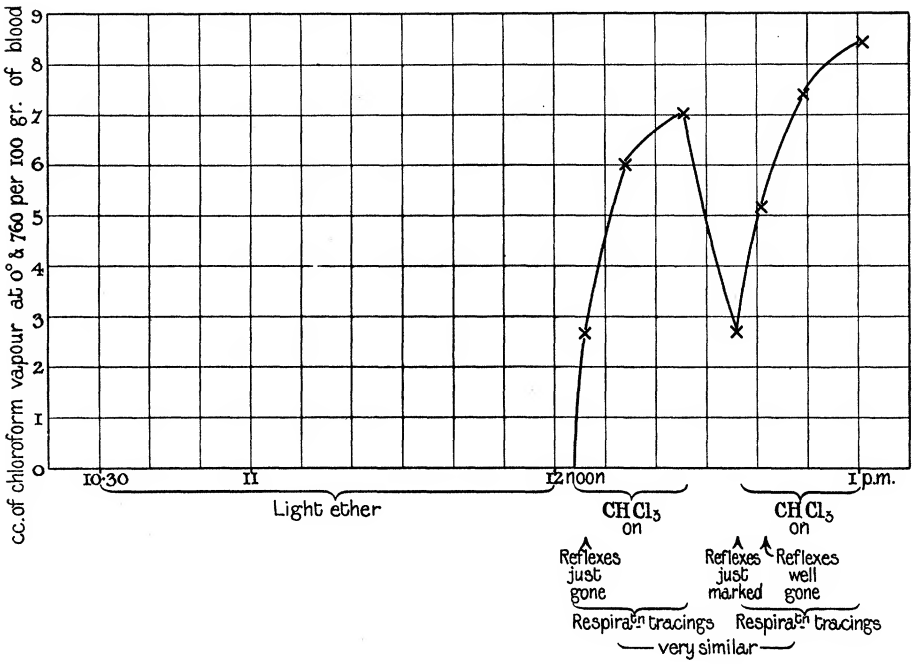
Experiment 8.—In this experiment a female cat, weighing 2·7 kilogrammes, was taken and the chloroform was administered from bags filled with air, containing approximately 2 per cent. of chloroform vapour, by means of the Dubois apparatus. The object of this experiment was to obtain two curves showing in the one case the proportions of chloroform in the blood at various stages up to the asphyxiation point immediately after anæsthetisation by ether, and in the other case the amounts at the various stages in a second chloroform experiment, after the animal had recovered from the first.

After the control sample had been taken under ether, the cat was allowed so far to recover that the reflexes were well marked, and then the chloroform was administered. Samples of blood were collected when the reflexes had

Table VIII.—Cat (female). Weight, 2·7 kilogrammes.

(In this experiment the chloroform was administered by Dubois apparatus, and was approximately 2 per cent. strength.)

Time.	Anæsthetic.	Weight of blood in gr.	Weight of AgCl.	Percentage of chlorine.	Difference from ether control—			Colour.	Respirations per minute.	Remarks.
					As chlorine.	As chloroform.	As vapour of $CHCl_3$ at 0° C. and 760 mm.			
A.M.										
10.30	Ether on, light anæsthesia	—	—	—	—	—	—	—	28	
11.58	.....	4·1885	0·0578	0·3402	—	—	—	Fairly bright	—	Reflexes just visible (eye and tail)
11.59	Chloroform on ...	—	—	—	—	—	—	—	22	
12.0	Chloroform off.									
P.M.										
12.3	,, ...	—	—	—	—	—	—	—	—	Reflexes marked
12.4	Chloroform on.									
12.6	,, ...	5·4454	0·0779	0·3526	0·0125	0·014	2·63	Very bright ...	24	Reflexes just gone.
12.14	,, ...	4·1395	0·0619	0·3686	0·0284	0·0319	6·0	Darker	22	Animal very deeply under, but breathing satisfactorily
12.26	,, ...	4·6527	0·0705	0·37352	0·0333	0·0374	7·03	Much darker	—	
12.27	Chloroform off.									
12.33	,, ...	—	—	—	—	—	—	—	34 (frequent, deep)	Eye reflexes just beginning
12.35	,, ...	—	—	—	—	—	—	—	—	Slight tail reflex appears
12.36	,, ...	5·1162	0·0732	0·3527	0·0125	0·01406	2·64	Bright.		
12.37½	Chloroform on.									
12.39	,, ...	—	—	—	—	—	—	—	—	Reflexes began to go
12.41	,, ...	4·7880	0·0708	0·3645	0·0243	0·0273	5·13	Darker	39	Reflexes completely gone
12.49	,, ...	6·3007	0·096	0·3756	0·0354	0·0398	7·46	Much darker...	40	Animal very deeply under, but breathing satisfactorily
1.1	,, ...	5·2791	0·0814	0·3801	0·0399	0·0448	8·42	About same ...	(shallow) 20	



D.

Constructed from Experiment 8. Samples of arterial blood (+).

just disappeared, when the animal was moderately deeply under, and when respiration was stopping. The animal was then allowed partially to revive, and a sample was taken when the reflexes were well marked. Chloroform was now administered again, and samples were collected when the reflexes had *just* disappeared, when the animal was deeply under, and when respiration began to cease, the various points at which the blood was taken being selected so as to correspond as far as we could judge with those in the first part of the experiment.

The results of the experiment are given in Table VIII and Curve D. It will be noticed that the two portions of the curve correspond in form, but the second part is slightly shifted in an upward direction.

Experiment 9.—The object of this experiment was to test the effect on the amounts of chloroform necessary to produce various anæsthetic phenomena of taking away a large proportion of the animal's blood, which is undoubtedly the vehicle through which chloroform is enabled to produce its effects on the animal. For this purpose a female cat, weighing 3·3 kilogrammes, was selected, and the chloroform was administered by means of a Woulff's bottle, in which the height of the air inlet tube above the chloroform could be varied readily. After the control sample of blood had been withdrawn, the animal

Table IX.—Cat. Weight, 3·3 kilogrammes.

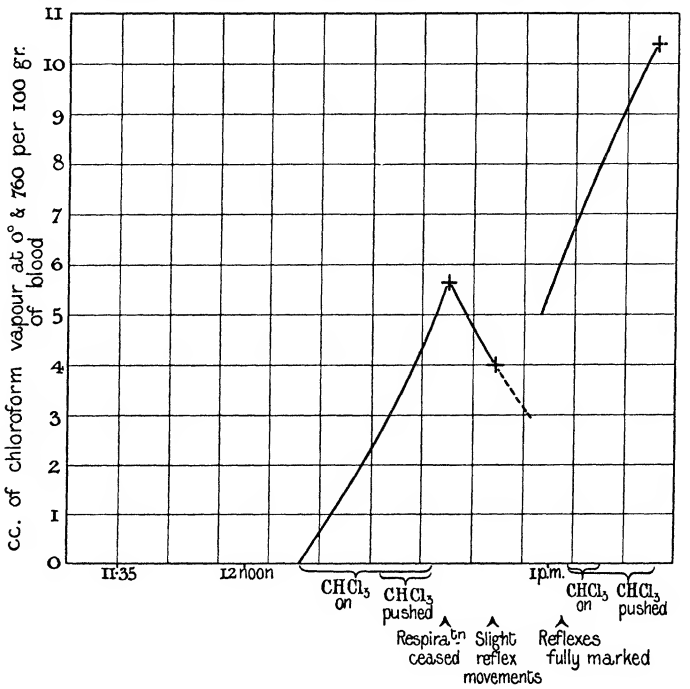
Time.	Anæsthetic.	Weight of blood in grammes.	Weight of AgCl.	Per-centage of chlorine.	Differences from control—			Colour.	Respira-tions per minute.	Remarks.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.			
A.M. 11.35	Ether put on.									
P.M. 12.5	Ether off.									
12.7	.....	—	—	—	—	—	—	—	28	
12.10	.....	—	—	—	—	—	—	—	—	
12.10½	.....	4·1749	0·0577	0·3407	—	—	—	Somewhat dark		Good reflexes
12.11	Chloroform on	—	—	—	—	—	—	—	28	
12.14	,, ...	—	—	—	—	—	—	—	36	
12.22	,, ...	—	—	—	—	—	—	—	—	Alternating rhythmical move-ments of hind limbs
12.24	,, ...	—	—	—	—	—	—	—	—	,, ,,
12.25	,, ...	—	—	—	—	—	—	—	—	,, ,,
12.27	Chloroform pushed.									
12.30	,, ,,	—	—	—	—	—	—	—	44	
12.33	,, ,,	—	—	—	—	—	—	—	52	
12.35	,, ,,	—	—	—	—	—	—	—	80	
12.37	,, ,,	—	—	—	—	—	—	—	16	,, ,,
12.40	Chloroform off	4·7989	0·0715	0·3673	0·0266	0·0299	5·61	Very dark	—	Animal revived naturally
12.42	.....	—	—	—	—	—	—	—	28	Respiration deep
12.46	.....	—	—	—	—	—	—	—	28	,,
12.47	.....	—	—	—	—	—	—	—	—	Reflexes began very slightly
12.49	.....	5·2056	0·076	0·3599	0·0192	0·0216	4·05	Bright red		
12.50	.....	—	—	—	—	—	—	—	—	32 c.c. blood withdrawn, making total since control of about 47 c.c.

12.52	....	—	—	—	—	—	—	—	24	Reflexes fully back
1.3	.....	—	—	—	—	—	—	—	—	
1.4	Chloroform on	—	—	—	—	—	—	—	24	Reflexes vanished
1.6	,, ...	—	—	—	—	—	—	—	24	
1.8	,, ...	—	—	—	—	—	—	—	28	
1.10	Chloroform pushed	—	—	—	—	—	—	—	32	
1.11	,, ,,	—	—	—	—	—	—	—	40	
1.13	,, ,,	—	—	—	—	—	—	—	—	Rhythmical alternate leg movements began
1.14	,, ,,	—	—	—	—	—	—	—	56	,, ,,
1.16	,, ,,	—	—	—	—	—	—	—	80	,, ,,
1.17	,, ,,	—	—	—	—	—	—	—	48	
1.18	,, ,,	—	—	—	—	—	—	—	64	Faint and shallow respiration
1.19	,, ,,	—	—	—	—	—	—	—	64	,, ,,
1.19½	,, ,,	—	—	—	—	—	—	—	—	Respiration stopped
1.21	,, ,,	5·6528	0·0894	0·3899	0·0492	0·0552	10·37	Very dark		

was allowed to recover until the ether anæsthesia was very light. The chloroform bottle was now connected to the Chauveau valve, and the anæsthetic administered fairly gently for 14 minutes, after which it was pushed by lowering the inlet air tube to the surface of the chloroform, and a sample of blood was collected when respiration ceased; the blood at this stage was very dark in colour. During the period in which the anæsthesia was pushed curious alternating rhythmical movements of the hind limbs were noticed. The cat was allowed to recover naturally, and a sample of blood collected when the reflexes were slightly marked. The colour of the sample was bright red. Thirty-two cubic centimetres of blood were now withdrawn from the animal, making in all since the control sample about 47 to 50 c.c. This quantity was probably a little over 1/3 of the total blood in the animal.

The chloroform was now administered again, and, after the first few minutes, was pushed until respiration stopped, when a sample, very dark in colour, was collected. During the later stages of this anæsthesia, the above-mentioned rhythmical movements of the limbs were again noticed, and they ceased a little before respiration stopped.

The results of this experiment are recorded in Table IX and Curve E. It will be noticed that the amount of chloroform in the blood at the asphyxia-



E.

Constructed from Experiment 9. Samples of arterial blood (+).

tion stage was very much higher in the second half of the experiment than in the first. In connection with this experiment we would draw attention to the results of the administration of chloroform recorded at the end of Tables I and II.

In the two following experiments we used dogs instead of cats. The phenomena observed were similar to those in the case of cats, but the percentages of chloroform found at the various stages were somewhat higher.

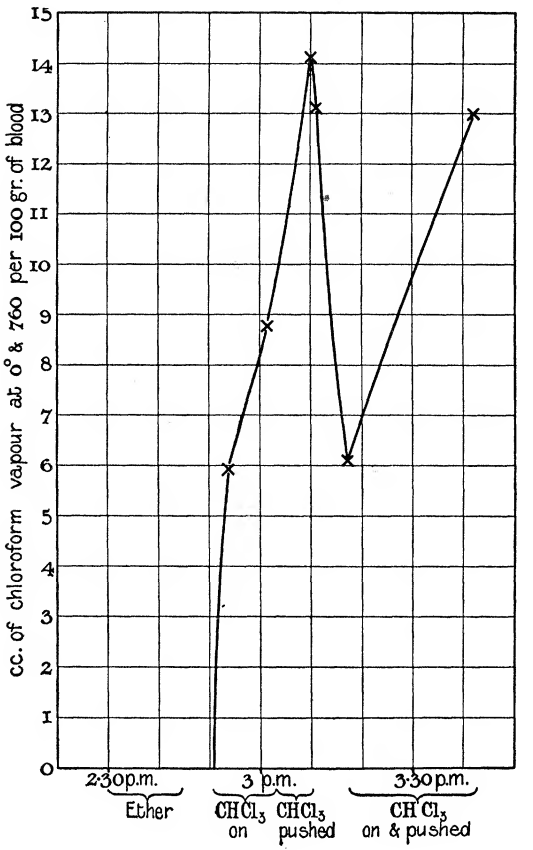
Experiment 10.—For the purpose of this experiment a dog weighing 10 kilogrammes was selected, and chloroform was administered by means of a Woulff's bottle, the temperature at the time being rather high—viz., 24° C., so that the percentage of chloroform in the air inhaled was probably somewhat higher than in those other experiments in which a Woulff's bottle was made use of. Blood samples were taken from the left carotid. The control sample was taken as usual under light ether anæsthesia, but in this case an extra sample of 10 c.c. was also withdrawn for another purpose. Chloroform was then administered, and samples of blood were collected when the reflexes had just gone and when the animal was deeply anæsthetised. At the latter stage an extra 10-c.c. sample was also taken for another purpose. The anæsthesia was then pushed by lowering the air inlet tube to the surface of the chloroform, and a sample of blood withdrawn when respiration ceased. The animal was now allowed to recover naturally, and samples were taken when breathing commenced regularly and when reflexes began to reappear. At the latter stage chloroform was again administered until the animal died, when a sample of blood was taken from the left auricle of the heart and analysed. The results of this experiment are recorded in Table X and Curve F′. As in the case of cats, the colour of the blood followed closely the percentage of chloroform in the blood.

Experiment 11.—In this case a very vigorous dog, weighing 10·5 kilogrammes, was selected, and the blood was withdrawn from the right carotid. The chloroform was administered from bags filled with mixtures of chloroform and air of known composition by the Dubois apparatus. The control sample of blood was taken under light ether anæsthesia, and at the same time a 10-c.c. sample for another purpose. Chloroform of 2-per-cent. strength was then administered, and duplicate samples taken when the reflexes had just gone. Ten minutes later another sample of blood was taken. At this stage the strength of the anæsthetic was increased to 3 per cent., and a sample of blood withdrawn eight minutes later. The strength of the chloroform was then increased to 4·5 per cent., and nine minutes later a duplicate sample of blood was withdrawn. Fourteen minutes later another sample was collected, and the chloroform was cut off. At this stage respiration *had not ceased*, but

Table X.—Dog. Weight, 10 kilogrammes. The chloroform was administered by means of a Woulff's bottle. Laboratory very warm, about 24° C. Left carotid tapped.

These figures were obtained by a less exact method than the others.

Time.	Anæsthetic.	Weight of blood in grammes.	Weight of AgCl.	Percentage of chlorine.	Differences from control—			Colour.	Remarks.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.		
P.M.									
2.30	Ether started.								
2.45	,, ...	4·7500	0·0532	0·2761	—	—	—	Medium shade	Sample of 10 c.c. taken extra
2.47	Chloroform on.								
2.49	,, off.								
2.51	Chloroform on	—	—	—	—	—	—	—	Respiration 67 per min.; reflexes just gone
2.54	,, ...	4·8576	0·0599	0·3040	0·0279	0·0313	5·87	Rather darker	Respiration 65; blood darker than previous sample
3.1	,, ...	3·7845	0·0487	0·3179	0·0418	0·0470	8·82	Darker	Extra sample 10 c.c. taken
3.4	Chloroform pushed	—	—	—	—	—	—	—	Respiration 17; weak
3.10	,, ,,	3·7673	0·0523	0·3430	0·067	0·075	14·1	Very dark	Breathing stopped, after gasping. Allowed to recover with air
3.10½	$CHCl_3$ off.								
3.11	.....	4·4874	0·0616	0·3384	0·0623	0·0699	13·1	Very dark	
3.17	$CHCl_3$ off	5·2033	0·0644	0·3051	0·0290	0·0325	6·1	Bright	Reflexes just appearing
3.17½	$CHCl_3$ on and pushed	—	—	—	—	—	—	—	Respiration 70
3.42	,, ,,	5·0958	0·0698	0·3377	0·0616	0·0691	12·98	Very dark	Animal died; sample taken from heart



F.

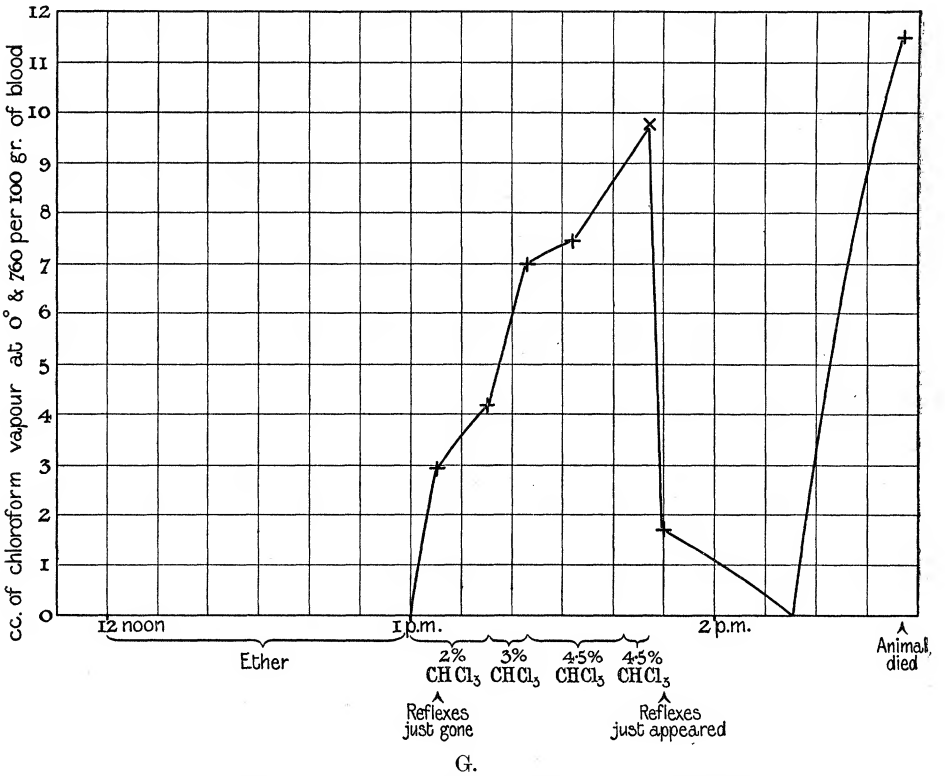
Constructed from Experiment 10. Samples of arterial blood (+).

was slow and shallow. During the later stages of the anæsthesia alternate twitchings of the paws were noticed, which, however, ceased as the asphyxiation point was approached. A sample of blood was collected when the reflexes again began to be quite evident, and to prevent the animal completely reviving a little ether was administered. Twenty-eight minutes after the chloroform had been cut off a sample of blood was collected, the analysis of which showed that the chloroform had been completely eliminated from the blood, and that the chlorine content had returned to normal. Chloroform was now administered rapidly by means of a Woulff's bottle, and a sample of the blood was collected after respiration ceased and as the animal was dying.

The results of the experiment are given in Table XI and Curve G.

Table XI.—Dog. Weight, 10·5 kilogrammes. Right carotid tapped. Chloroform given by Dubois.

Time.	Anæsthetic.	Weight of blood in gr.	Weight of AgCl.	Per-centage of chlorine.	Difference from control—			Colour.	Remarks.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.		
12 noon	Ether began.								
P.M.									
12.55	Very light ether	4·8512	0·058	0·2947	—	—	—	Bright	Reflexes visible; 10 c.c. blood taken for another purpose
1.0	Chloroform on, 2 per cent.								
1.4	,, ,,	5·3664	0·0672	0·3087	0·0140	0·0157	2·95	Fairly bright.....	Reflexes just gone; 10 c.c. blood taken
1.15	,, ,,	4·7210	0·0602	0·3143	0·0196	0·022	4·14	Scarcely darker	
1.15½	3 per cent. $CHCl_3$ on.								
1.23	,, ,, ...	6·0238	0·0801	0·3278	0·0331	0·0372	6·98	Darker	
1.23½	4·5 per cent. $CHCl_3$ on.								
1.32	,, ,,	4·6429	0·0622	0·3302	0·0355	0·0399	7·49	Very dark	Breathing slow and shallow, but regular; muscular movements of tail began; 10 c.c. extra blood taken
1.37	,, ,,	—	—	—	—	—	—	—	Alternate twitching of fore paws began
1.41	,, ,,	—	—	—	—	—	—	—	These movements more marked
1.43	,, ,,	—	—	—	—	—	—	—	Respiration very shallow
1.46	,, ,,	5·6088	0·0776	0·341	0·0463	0·0521	9·77	Very dark indeed	Animal still breathing
1.46½	Chloroform off	—	—	—	—	—	—	—	Began to breathe rapidly; tail movements ceased
1.48	,,								
1.50	,, ...	4·5619	0·056	0·3026	0·0079	0·0089	1·66	Very bright	Reflexes began to be quite evident
1.52	Slight ether on.								
2.0	Slight ether on	—	—	—	—	—	—	—	Respiration quite regular and satisfactory
2.15	,, ...	7·0214	0·0832	0·2921	−0·0026				
2.25	$CHCl_3$ on, using Woulff's bottle, with air bubbling through $CHCl_3$.								
2.37	,, ,,	5·605	0·0794	0·3492	0·0545	0·0612	11·48	Very deep indeed	Animal dying



G.

Constructed from Experiment 11. Samples of arterial blood (+).

General Conclusions.

No previous observations on cats are available for reference; all the observers whose work has been considered in the earlier part of this paper carried out their investigations exclusively on dogs.

It is difficult in any case to ascertain the exact moment at which an animal is really anæsthetised. In our experiments we have taken the moment of the disappearance of both conjunctival reflexes as a fixed point, though the determination of this presents some difficulties, since the reflex may disappear from one eye a minute earlier than in the other. The cessation of the tail-reflexes may sometimes precede, sometimes succeed that of the conjunctiva. The times when samples of blood were taken in our experiments at this stage of anæsthesia slightly differ; for example, blood was withdrawn sometimes when the conjunctival reflexes definitely disappeared, sometimes when they definitely reappeared, sometimes at a point when they had almost vanished or had returned in one eye.

Combining the results of the experiments described, which form only a

portion of a much larger number of observations, some of which were incomplete and only partly serviceable from the inevitable bursting of tubes, which may occur in carrying out a series of Carius' determinations for chlorine, the results of our experiments show that the amount of chloroform in arterial blood of cats at the stage of disappearance of conjunctival reflexes varies between 14 and 27·6 milligrammes per 100 grammes of blood. This amount varies with the individual animal, a fact noticed by others who have worked with dogs. This probably depends on the condition of the animal in the widest sense of the term; thus some were male, some female, and we were also in many cases ignorant whether the animals had been recently fed or the opposite. The ages of the animals were also unknown. Reference to the tables shows that body-weight is without influence on the percentage of chloroform in the blood necessary to produce anæsthesia.

An examination of Curves A, B, C, and D, shows that the rate of induction of anæsthesia varies for different individuals. This does not appear to be altogether dependent upon the percentage of chloroform in the inspired air, but is a feature peculiar to each individual animal. It can be seen from Curve D that the two upward slopes of the curve are closely similar in form in the same animal. In this curve, constructed from an experiment during which a constant percentage of chloroform was inhaled, the lethal point is raised during the second anæsthetisation—in fact, all the corresponding points of the second slope are higher than for the first. The explanation of this, which is a feature we have often noticed, might be that the first anæsthetisation is assisted by some ether which still remains in the animal. But this supposition is not confirmed by a consideration of Curves B and C. We are inclined to attribute the shifting-up of the second part of the curve and the raised lethal point to the loss of blood taken during the course of the experiment, as more blood was abstracted from the animal in Curve D than in experiments Curves B and C. The effect of removal of blood markedly alters the lethal dose in blood, a fact made quite evident in Curve E. However, at this stage of our work we do not propose to offer the suggestion that loss of blood is the only factor which raises the lethal dose, as we are still engaged in experiments devised to ascertain the progress of chloroform anæsthesia in animals under varied physiological conditions.

The lethal dose which arrests respiration in cats is also variable, and averages about 40 milligrammes per 100 grammes of blood, and it will be seen from the Tables that a narrow margin exists between the weight of chloroform in blood at the moment of anæsthesia (loss of conjunctival reflexes) and cessation of respiration. The lethal dose of Curves B and C is slightly higher in the second anæsthetisation than in the first

We are inclined to attribute this to slight excess of chloroform in the inhaled air.

After anæsthesia the chloroform is eliminated with extreme rapidity. The rate of elimination varies in different animals, but the rate of disappearance of chloroform is far more constant than the rate of assumption.

In the two observations quoted for dogs the results are not quite similar in character to those observed in cats, for the lethal dose and the quantity of chloroform required to produce various stages of anæsthesia are somewhat higher. The rapidity of elimination of chloroform appears to be even greater than in the case of cats. The figures we have obtained for dogs are in fair agreement with those of the French observers. Especially is this the case for the lethal dose, but the difference noticed for the anæsthetic dose depends probably on the criterion of anæsthesia adopted. The mean value in the arterial blood of dogs is given in following Table, though it must be pointed out that they used much heavier animals than those at our disposal.

Table XII.

	Tissot.	Nicloux.	Our experiments, reflexes just gone.
	milligrammes.	milligrammes.	milligrammes.
Anæsthesia	34—40	50	16—31
Lethal dose.....	59—105	69—73	61—69

II.—*Experiments to ascertain how the Chloroform distributes itself between the Corpuscles and the Plasma.*

In the experiments on this subject the animals were anæsthetised and the samples of blood were collected in exactly the same way as in the series already described. The samples were in most experiments mixed with a saturated solution of sodium oxalate, in the proportion of 0·5 c.c. oxalate solution to 10 c.c. of blood, to prevent clotting. They were then centrifugalised and the plasma separated from the red sediment by means of a fine pipette.

In other experiments 10 c.c. samples of blood were withdrawn from the animal, mixed with the oxalate and divided into two equal volumes before centrifugalisation. In a few experiments the blood was cooled by ice and centrifugalised in an ice jacket without the addition of oxalate. Following this plan it was found very difficult to get a satisfactory separation of the red corpuscles before clotting took place. We found that this could be effected

much more easily in the case of cat's blood than in the case of dog's. The samples of corpusles and plasma were analysed in the same way as before.

With the centrifugal machines at our command we found it very difficult to get exactly equal ratios of separation of red matter and plasma in the control samples taken under ether and the samples taken under chloroform. As the percentage of natural chlorine in the red corpuscles is very different from that in the plasma it is obviously impossible to calculate with any degree of accuracy from the experimental results the exact distribution of chloroform between the corpuscles and plasma, unless the ratios of separation in the control and chloroform samples are sensibly equal. With very great care we succeeded in getting a sufficiently equal separation in a few experiments to permit of this calculation being performed with accuracy. In the other experiments, though the calculation could not be made, the results obtained indicated clearly that the chloroform associated itself with the corpuscles rather than the plasma.

Experiment 12.—In this experiment a cat was anæsthetised in the way described, using a Woulff's bottle. Samples of blood were collected under ether, and also when the animal was very deeply under the influence of chloroform. Clotting was prevented by oxalate. Unfortunately the Carius tubes containing the corpuscles exploded, but the percentages of chlorine found in the control sample and the chloroform sample were respectively 0·3819 and 0·3895. This was one of our earlier experiments, and the silver chloride was separated by filtration through paper and weighed in the usual manner instead of by the method described at the beginning of this paper; the results are, therefore, approximate, and we give them for what they are worth. They show, however, that little, if any, of the chloroform administered was in the plasma.

Experiment 13.—For the purpose of this experiment a cat weighing 2·8 kilogrammes was taken, the chloroform being administered by means of a Woulff's bottle, and the blood collected when the animal was deeply under. The samples of blood were centrifugalised in ice without the addition of oxalate. The ratios of separation in the control and chloroform samples were not, however, equal. The chlorine was estimated by the method used in the previous experiment, so that we do not regard the analysis as possessing the highest degree of accuracy.

The following Table XIII (p. 447) gives the result obtained.

Whereas the percentage of chlorine in the corpuscles shows an increase of 0·056, that in the plasma is only 0·005.

Experiment 14.—In this experiment a dog was anæsthetised with ether and a control sample of blood taken. Chloroform was then administered by

Table XIII.

	Weight of corpuscles.	Weight of plasma.	Weight of silver chloride from corpuscles.	Weight of silver chloride from plasma.	Percentage of chlorine.	
					In corpuscles.	In plasma.
Control expt., under ether	2·7095	2·8975	0·0342	0·0496	0·315	0·423
Expt., under chloroform	1·7798	3·4678	0·0268	0·0587	0·371	0·428

means of a Woulff's bottle, and three samples of blood collected at various stages of anæsthesia up to the asphyxiation point. An attempt was made to centrifugalise the blood simply cooled in ice without the addition of oxalate, but all the samples clotted. They were, therefore, allowed to stand until contraction took place, when they were again centrifugalised, and the corpuscles and serum analysed. The analysis was made by the same method as in Experiment 13. The ratios of separation of corpuscles and serum in the various samples were not equal, so that ratios of chloroform distribution could not be calculated. The results, however, indicate that practically no chloroform went into the plasma, except when the anæsthesia was pushed to an extreme point. The results are given in the following Table.

Table XIV.

	Weight of corpuscles.	Weight of silver in corpuscles.	Percentage of chlorine in corpuscles.	Weight of serum.	Weight of silver chloride in serum.	Percentage of chlorine in serum.
Control samples under ether	3·5942	0·0376	0·254	2·042	0·0321	0·3875
Under $CHCl_3$ when reflexes gone	3·3118	0·0378	0·282	2·258	0·0355	0·3876
Under $CHCl_3$ when breathing was very feeble	2·8333	0·0367	0·319	2·2084	0·0373	0·4173

Experiment 15.—In this experiment a cat weighing 3·1 kilogrammes was taken and anæsthetised with ether; 10 c.c. of blood were then withdrawn when the reflexes were scarcely marked, and mixed with $\frac{1}{2}$ c.c. of saturated sodium oxalate. The sample was then divided into two equal portions, each of which was weighed and centrifugalised. One sample was analysed and

the other left for experiments which will be mentioned later; the animal was then allowed to recover partially, and chloroform was administered slowly by means of a Woulff's bottle. Samples were collected when the reflexes had just gone, and when respiration was on the point of ceasing. The samples were treated in the same way as the control sample. The analyses were made with the utmost care. The results are recorded in Table XV.

It will be noticed that the percentage of chlorine in the plasma remained constant, at any rate to within the probable errors of experiment. Calculation shows that 71 and 73 per cent. of the chloroform was taken up by the red corpuscles. Probably, however, a much higher proportion than this, perhaps the whole, was taken by the corpuscles, as the percentage of chlorine in the plasma remained the same. The discrepancy can, we think, be quite accounted for by the fact that the ratios of separation of corpuscles and plasma in the various samples were not quite equal.

Experiment 16.—In this experiment the samples of blood used were taken from the dog used in Experiment 10, chloroform being administered by the Dubois apparatus. The results are given in Table XVI.

Here again the bulk of the chloroform was absorbed by the red matter, and little went into the plasma. Calculation shows that at the point of the vanishing of the reflexes 64 per cent. attached itself to the corpuscular matter,

Table XV.—Cat.

Time.	Anæsthetic.	Weight of blood + 0·25 c.c. oxalate of soda.	Weight of AgCl.	Total percentage of chlorine.	Difference from control—			Weight of corpuscles.	Weight of AgCl.	Percentage of chlorine in corpuscles.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.			
A.M.										
10.45	Ether began.									
11.20	Ether.....	5·1975	0·0685	0·3249	—	—	—	1·9781	0·0184	0·2293
11.20	$CHCl_3$ on.									
11.34	„	5·1823	0·0719	0·3420	0·01714	0·01924	3·6	1·9439	0·0209	0·2650
11.40	$CHCl_3$ pushed.									
11.46	„	—	—	—	—	—	—	—	—	—
11.53	„	—	—	—	—	—	—	—	—	—
11.54	„	5·2267	0·0760	0·35844	0·0336	0·0377	7·07	1·8804	0·0237	0·3107

Percentage of chloroform taken by corpuscles
„ „ „ „

but in this case the calculation is vitiated even more than in the previous experiment by inequality in the ratios of separation, which were 1·034 in the control and 1·048 in the chloroformed sample later.

Experiment 17.—For the purpose of this experiment a cat weighing 3·5 kilogrammes was taken. A control sample of 10 c.c. was taken and mixed with ½ c.c. sodium-oxalate solution and centrifugalised. The cat after partial recovery was then slowly chloroformed, using bags filled with air containing approximately 2 per cent. of chloroform vapour, by means of the Dubois apparatus. A 10 c.c. sample of blood was collected when the animal was very deeply under the influence of the anæsthetic. The colour of the sample was very dark. It was treated exactly as in the case of the control. The samples, which were larger than in other experiments, were analysed with the utmost precaution. In this case we were fortunate enough to get a very good separation of corpuscles and plasma, and the ratios in the case of control and chloroform samples were very nearly equal.

The results are given in Table XVII. In this experiment the whole of the chloroform associated itself with the red corpuscles.

A number of experiments were performed, the results of which are not recorded, as, owing to accidents in the analysis, they did not form a complete series. The results obtained, however, were in accordance with those quoted.

Weight, 3·1 kilogrammes.

Percentage of corpuscular chlorine per cent. of blood.	Difference of corpuscular chlorine per cent. of blood.			Weight of plasma.	Weight of AgCl.	Percentage of chlorine in plasma.	Remarks.
	As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.				
0·0873	—	—	—	3·2194	0·0501	0·3836	Sample taken when reflexes just present. Respiration 32 per min.
0·0994	0·0121	0·0136	2·56	3·2384	0·0510	0·3882	Sample taken when reflexes had just completely gone. Respiration 44 per min.
—	—	—	—	—	—	—	Respiration 28 per min.
—	—	—	—	—	—	—	Respiration 48, and very shallow
0·1118	0·0245	0·0275	5·17	3·3463	0·0523	0·3853	Respiration 20, and very shallow. Colour of blood very dark

71·1, at period of vanishing of reflexes.
73·1, at period of asphyxiation.

Table XVI.—Dog. Weight, 23 lbs.

Time.	Anæsthetic.	Weight of blood + 0·25 c.c. oxalate of soda.	Weight of AgCl.	Total percentage of chlorine.	Difference from control—			Weight of corpuscles.	Weight of AgCl.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.		
12 noon	Ether began.								
P.M.									
12.55	Very light ether.....	4·8812	0·0544	0·27473	—	—	—	2·3997	0·0194
1.0	Chloroform on, 2 per cent.								
1.4	„ „	5·0918	0·0601	0·29096	0·0162	0·0182	3·42	2·4859	0·0224
1.15	3 per cent. on.								
1.23	4·5 per cent. on.								
1.32	.....	*	*	*	*	*	*	2·4743	0·0267

* These values could not be calculated, owing to Percentage of chloroform taken by corpuscles

Table XVII.—Cat.

Time.	Anæsthetic.	Weight of blood + 0·5 c.c. oxalate of soda.	Weight of AgCl.	Total percentage of chlorine.	Difference from control—			Weight of corpuscles.	Weight of AgCl.	Percentage of chlorine in corpuscles.
					As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.			
P.M.										
3.0	Ether began.									
3.41	Ether on	10·1997	0·1340	0·32385	—	—	—	3·9132	0·0386	0·2431
3.42	Ether off.									
3.47	$CHCl_3$ on, 2 per cent.									
4.30	„ „	10·0375	0·1400	0·34382	0·01997	0·0224	4·2	3·8050	0·0460	0·298

Percentage of total chlorine

Conclusions.—It would appear from these experiments that in cats the chloroform associates itself primarily with the red corpuscles, and never gets into the plasma unless the anæsthesia is pushed to an extreme point and the anæsthetic is rapidly administered. This conclusion also applies to dogs, but in this case the chloroform appears to enter the plasma somewhat more readily on pushing the anæsthetic. This conclusion is also in agreement with the results of Nicloux.

Chloroformed with Dubois apparatus.

Percentage of chlorine in corpuscles.	Percentages of corpuscular chlorine per cent. of blood.	Difference of corpuscular chlorine per cent. of blood.			Weight of plasma.	Weight of AgCl.	Percentage of chlorine in plasma.	Remarks.
		As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.				
0·1993	0·09797	—	—	—	2·4815	0·0350	—	Reflexes just marked
0·2221	0·10844	0·0105	0·0118	2·2	2·6059	0·0377	0·3566	Reflexes gone
0·266	*	*	*	*	2·1102	0·0312	0·3636	Very deeply marked; blood very dark

‡ a few drops of the total plasma having been lost.
- at period of vanishing reflexes, 64·3.

Weight, 3·5 kilogrammes.

Percentage of corpuscular chlorine per cent. of blood.	Difference of corpuscular chlorine per cent. of blood.			Weight of plasma.	Weight of AgCl.	Percentage of chlorine in plasma.	Colour.	Remarks.
	As chlorine.	As $CHCl_3$.	As vapour of $CHCl_3$ at 0° C. and 760 mm.					
0·09329	—	—	—	6·2865	0·0954	0·37409	Bright	Sample of 10 c.c. taken
0·11297	0·0197	0·0221	4·15	6·2325	0·0940	0·37179	Very dark	Sample of 10 c.c. taken

taken by corpuscles, 98·5.

The chloroform in the blood drawn from an anæsthetised animal appears to be very firmly held, as in none of the samples examined did we notice any marked smell of chloroform, and in some experiments blood allowed to stand over night suffered no diminution in chlorine.

In the hope of throwing some light on this question, duplicate samples of blood were withdrawn from an animal and centrifugalised. One series of samples were analysed directly and the other series after drying

in vacuo over sulphuric acid, and finally heating for some hours in the steam oven.

Experiment 18.—In this experiment the samples were taken from the dog used in Experiment 10. The chlorine values obtained by direct analysis without drying are those quoted in Experiment 16.

In the following Table we give the percentages of chlorine found in the undried and dried samples of corpuscles and plasma:—

	Percentage of chlorine.					
	Corpuscles.			Plasma.		
	Undried.	Dried.	Difference.	Undried.	Dried.	Difference.
Control samples under ether	0·1993	0·1811	0·0182	0·3477	0·3284	0·0193
Sample under $CHCl_3$ when reflexes had gone	0·2221	0·1885	0·0336	0·3566	0·3223	0·0343
Sample under $CHCl_3$ when blood was very dark in colour	0·2660	0·2027	0·0633	0·3636	0·3188	0·0448

We were surprised to find that the control sample both of corpuscles and plasma lost chlorine on drying; this loss was more marked in the case of the plasma. An inspection of the Table, however, will show that the chloroform samples lost considerably more chlorine, and that the figures obtained after drying are almost identical in each set. All the chloroform therefore appeared to be eliminated on drying.

Experiment 19.—The results quoted in this experiment were obtained with the blood of the animal mentioned in Experiment 15, which was treated in the same manner as described in Experiment 18, but the heating was not so prolonged, and the temperature was not above 90°.

	Percentage of chlorine.					
	Corpuscles.			Plasma.		
	Undried.	Dried.	Difference.	Undried.	Dried.	Difference.
Control under ether ...	0·2293	0·2297	—	0·3836	0·3511	0·0325
$CHCl_3$ when reflexes gone	0·2650	0·2486	0·0164	0·3882	0·3518	0·0364
$CHCl_3$ very deeply under	0·3107	0·2854	0·0253	0·3852	lost	

In this case the corpuscle control suffered no loss, but that of the plasma was marked. Possibly this may be due to the fact that in this experiment the separation of the corpuscles and plasma was much more complete. In this case the chloroform was not completely eliminated from the corpuscles. The loss suffered by the control and chloroform plasma was practically the same, so that probably little or no chloroform was present.

Experiment 20.—In order to prove conclusively that the loss of chlorine noted in the control samples in the two previous experiments was really due to the evolution of free hydrochloric acid on heating, a cat was anæsthetised with ether, and bled. About 18 grammes of the blood were mixed with a saturated solution of sodium oxalate (neutral) in the proportion of 1 c.c. to 47 of blood and centrifugalised. 12·9 grammes of plasma and 4·9 grammes of corpuscles were obtained. The samples were then placed in desiccators and dried *in vacuo* over sulphuric acid at the temperature of the laboratory. In each desiccator was suspended a small tray of pure crystals of copper sulphate to absorb any hydrochloric acid that might be evolved. After the samples were dry the copper sulphate crystals in each case were dissolved in water and tested for chlorine. We were, however, unable to detect with certainty any trace of hydrochloric acid. It must be remembered, however, that the quantities evolved from the amounts of blood taken would be in any case exceedingly small. The dried samples of plasma and corpuscles were then placed in small flasks. Each flask was connected with a larger flask, partly filled with strong sulphuric acid, by means of a fairly wide tube containing crystals of pure copper sulphate, and the air was pumped from the apparatus. The flasks containing the dried matter were then heated for four or five hours on the water bath. At the end of the experiment, the copper sulphate crystals in each tube were dissolved in water and tested for chlorine.

Each solution was found to give a slight but quite definite precipitate with silver nitrate, which careful tests proved to be silver chloride. The precipitate appeared to be somewhat larger in the case of the plasma than in the case of the corpuscle. The precipitates were, however, too small to admit of being accurately weighed.

From these experiments it is therefore evident that dried blood loses traces of chlorine at temperatures between 80° and 100° C.

It is somewhat difficult to explain these rather unexpected results, but it may be that the loss of hydrochloric acid is due to the interaction of the sodium phosphate with the sodium chloride of the blood at the temperature of the experiment. This hypothesis is in accordance with the fact which we have before noticed that mixtures of common phosphate of soda and salt lose

a slightly greater weight on drying than can be accounted for by loss of water, either by crystallisation or hydration.

In order to subject it to a still further test, 0·5 gramme of purified phosphate of soda was mixed with 10 grammes of pure salt—these being the approximate proportions in which these salts are stated to be present in blood—and the mixture was heated on the bath water for several hours in similar apparatus to that used in our experiments on blood. The copper sulphate was then dissolved in water and the chlorine present was estimated; 0·0805 gramme of silver chloride was obtained. Had the reaction taken place according to the following equation and gone to completion:—

$$Na_2HPO_4 + NaCl = Na_3PO_4 + HCl,$$

0·2 gramme of silver chloride should have been obtained. The experiment was repeated by a student in the laboratory of one of us with similar results.

These results appear to us to be of some interest in connection with Maly's theory as to the production of free hydrochloric acid in the organism.

We are engaged at present in further experiments based on the same plan of chlorine estimation, with a view to ascertaining the cause of anæsthesia under varied physiological conditions, some of the results of which we shall shortly be in a position to publish.

We take this opportunity of expressing our thanks to the Government Grant Committee of the Royal Society for assistance in carrying out this work.